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Rigid Fixation of the Spinal Column Improves Scaffold Alignment and Prevents Scoliosis in the Transected Rat Spinal Cord

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Abstract

Study Design—A controlled study to evaluate a new technique for spinal rod fixation after spinal cord injury in rats. Alignment of implanted tissue-engineered scaffolds was assessed radiographically and by magnetic resonance imaging.

Objective—To evaluate the stability of implanted scaffolds and the extent of kyphoscoliotic deformities after spinal fixation.

Summary of Background Data—Biodegradable scaffolds provide an excellent platform for the quantitative assessment of cellular and molecular factors that promote regeneration within the transected cord. Successful delivery of scaffolds to the damaged cord can be hampered by malalignment following transplantation, which in turn, hinders the assessment of neural regeneration.

Methods—Radio-opaque barium sulfate-impregnated poly-lactic-co-glycolic acid scaffolds were implanted into spinal transection injuries in adult rats. Spinal fixation was performed in one group of animals using a metal rod fixed to the spinous processes above and below the site of injury, while the control group received no fixation. Radiographic morphometry was performed after 2 and 4 weeks, and 3-dimensional magnetic resonance microscopy analysis 4 weeks after surgery.

Results—Over the course of 4 weeks, progressive scoliosis was evident in the unfixed group, where a Cobb angle of $8.13 \pm 2.03^\circ$ was measured. The fixed group demonstrated significantly less scoliosis, with a Cobb angle measurement of $1.89 \pm 0.75^\circ$ ($P = 0.0004$). Similarly, a trend for less kyphosis was evident in the fixed group ($7.33 \pm 1.68^\circ$) compared with the unfixed group ($10.13 \pm 1.46^\circ$). Quantitative measurements of the degree of malalignment of the scaffolds were also significantly less in the fixed group ($5 \pm 1.23^\circ$) compared with the unfixed group ($11 \pm 2.82^\circ$) ($P = 0.0143$).

Conclusion—Radio-opaque barium sulfate allows for visualization of scaffolds *in vivo* using radiographic analysis. Spinal fixation was shown to prevent scoliosis, reduce kyphosis, and reduce

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scaffold malalignment within the transected rat spinal cord. Using a highly optimized model will increase the potential for finding a therapy for restoring function to the injured cord.

Keywords

spine fixation; transection spinal cord injury; scaffold; scoliosis

Complete spinal cord transection provides a valuable model for determining the cellular and molecular factors necessary for promoting axonal regeneration. Although contusion and compression models mimic the types of injury that occur most frequently in patients,¹ interpretation of results using these models can be complicated by the occurrence of axonal regeneration and sprouting from distal segments of surviving axons.

The use of biodegradable scaffolds in transection injuries allows separate manipulation of the 3-dimensional structural, cellular, and molecular environment of the regenerating cord. An 85:15 polylactic acid: poly-lactic-co-glycolic acid (PLGA) scaffold has been shown to maintain its structural integrity for at least 8 weeks and starts to degrade significantly by 24 weeks, thereby providing an adequate time-frame for axonal regeneration.² This type of scaffold has been used in nerve and bone repair and in brain with few adverse effects.³⁻⁶ Biodegradable polymer inserts have also been extensively used in animal models to promote axonal regeneration in the peripheral nervous system,⁷⁻¹⁰ which has subsequently led to the development of suitable models for the central nervous system.^{2,11,12} Axonal regeneration was demonstrated through channels created in PLGA scaffolds that were loaded with Schwann cells and inserted into the spinal cord.² However, the resulting weakness that occurs at and below the level of transection can lead to the development of kypho-scoliosis in a significant number of animals. This, in turn, can compromise the alignment and stability of the implanted scaffold, which can hinder interpretation of the resulting axonal regeneration and functional recovery.

Spinal stabilization offers a solution to this problem. Fixation of the vertebral column has been shown to improve neurologic function in clinical spinal cord surgery.¹³ However, many animal models of SCI currently do not incorporate a fixation procedure, despite its reported benefits.¹⁴

This study was undertaken to assess the effect of a novel method of spinal fixation on scoliosis, kyphosis, and scaffold alignment in an animal transection model of SCI. Radiographic and 3-dimensional-magnetic resonance microscopy (MRM) analysis were used to quantify the degree of spinal curvature and malalignment of the scaffold within the cord. Radiographic visualization of the scaffold within the cord was achieved by radio-opaque barium sulfate impregnation. The barium sulfate did not interfere with MRM imaging.

Materials and Methods

Fabrication of Scaffolds

Scaffolds were fabricated from 85:15 PLGA by an injection molding-solvent evaporation process as previously described.²

Preparation of Scaffolds for Surgeries

The day before surgeries, scaffolds were impregnated with Radio-opaque barium sulfate to allow for visualization of the scaffold using radiographic analysis.

Surgery and Postoperative Care

All procedures involving animals were approved by the Institutional Animal Care and Use Committee at the Mayo Clinic. Adult female Sprague Dawley rats (200 g–275 g) were anesthetized with 60 mg/kg ketamine (Fort Dodge Animal Health, Fort Dodge, IA) and 2.5 mg/kg xylazine (Ben Venue Laboratories, Bedford, OH) by intraperitoneal (IP) injection. Akwa Tears lubricant ophthalmic ointment (Akron Inc., Buffalo Grove, IL 60089) was applied to the eyes to prevent drying. Hair was removed from the animals back (above the Cervical and thoracic vertebrae) using an electric clippers. Heating pad temperature was maintained at 37°C during the surgery and as needed for 24 hours post surgery. The operation was done using sterile technique and with the aid of a Zeiss OPMI-6 surgical microscope under a 3× magnification. A 4 cm midline incision was made along the T7–T10 spinous processes using a No. 10 scalpel blade. The thoracolumbar fascia and paraspinal musculature were incised along the spinous processes. A T9–T10 laminectomy was performed, the dura opened, and the cord transected using a No. 11 blade. The scaffold implant was placed in the transection gap and aligned to assure good contact and no blood clots at the rostral and caudal cord-scaffold interfaces (Figure 1A).

Surgeries were performed on 18 rats, 9 of which received spinal fixation, with the other 9 receiving no fixation (control group). Fixation was achieved by boring holes through the base of the spinous processes (2 above and 2 below the site the injury) using a micro drill. Steel wires (0.013 inches in diameter) were passed through these holes and a 1 mm diameter stainless steel rod was placed over the wires (Figure 1B). The wires were then tightened at each point to ensure stabilization of the rod against the dorsal aspect of the spinal column (Figure 1C).

After surgery, animals were given buprenorphine 0.05 mg/kg subcutaneously for pain for the first 3 to 4 days, Lactated Ringer's solution as needed, and Gentamicin (Schering-Plough, Union, NJ) intramuscularly for 3 to 7 days to prevent urinary tract infection. For the duration of the experiment, bladder expression and observation of the animals was performed twice daily.

Radiographic Imaging

Radiographic imaging of the animals was preformed immediately, 2 weeks and 4 weeks post surgery using a Philips Mammo Diagnost UC with 30 kV and 12 mA settings. Cobb angle measurements were taken on anterior-posterior radiographic projections of the spine. To minimize the variability of Cobb angle measurements, radiographs of each animal were remeasured with the same device and by the same person.¹⁵

Euthanasia and Harvesting of Tissue

Four weeks post surgery, animals were sacrificed with pentobarbital (Fort Dodge Animal Health, Fort Dodge, IA) intramuscularly and fixed by transcardial perfusion with 4% paraformaldehyde. The spinal column was removed en bloc and postfixed in 4% paraformaldehyde for 48 hours followed by removal of the spinal cord and further fixation in 4% paraformaldehyde for 24 to 48 hours.

Three-Dimensional MRM Analysis

Three-dimensional-MRM analysis was preformed on sagittal, axial, and coronal sections of the spinal cord containing the scaffold in both groups. Spinal columns were placed into 10 mm bore glass tubes for MRI analysis using the 7 Tesla vertical bore magnet with Bruker Biospin 10 mm MICRO imaging probe and ParaVision 3.0.2 image acquisition software. The degree of scaffold malalignment was quantified by drawing a line through the vertical axis of the cord and measuring the angle between this and the longitudinal axis of the scaffold.

Statistical Analysis

Student unpaired *t* test was used to analyze the degree of kyphosis, scoliosis, and scaffold malalignment. *P* values <0.05 were considered statistically significant. Graph Pad Prism 4 software was used for all analyses.

Results

Surgical Results

Surgery and postoperative care was completed without mortality. In one animal the rod slipped beneath the retaining wires and came to rest between L2 and L5. This was identified in the postoperative radiographs and the results from this animal were excluded from the analysis.

Radiographic Analysis

Radiographical analysis of the unfixed cords revealed progressive scoliosis at both 2 weeks (Figure 2B) and 4 weeks (Figure 2C) post surgery compared with immediately after the surgery (Figure 2A). Radiographical analysis of the animals with rod fixation generally showed that the scaffold was well-aligned within the cord (Figure 3A) with minimal scoliotic deformity (Figure 3B).

The mean scoliosis Cobb angle measurement between T6–T10 was $8.13 \pm 2.03^\circ$ in the unfixed group and $1.89 \pm 0.75^\circ$ in the fixed group (Figure 4A). The difference was statistically significant, with a *P* value of 0.0004.

The mean kyphotic angle Cobb angle measurement between T5–L5 showed a trend toward an improvement in the fixed group ($7.33 \pm 1.68^\circ$) compared with the unfixed group ($10.13 \pm 1.46^\circ$) (Figure 4B).

Three-Dimensional MRM Analysis

Three-dimensional-MRM analysis was performed on sagittal, axial, and coronal sections of the spinal cord containing the scaffold in both groups. Coronal (Figure 5A and B) and axial (Figure 5C and D) sections revealed greater alignment of the scaffold in the fixed group (Figure 5A and C) compared with the unfixed group (Figure 5B and D).

Quantitative measurements of the degree of scaffold malalignment (obtained from sagittal and coronal 3-dimensional-MRM images) revealed that the degree of malalignment was significantly less in the fixed group ($5 \pm 1.23^\circ$) compared with the unfixed group ($11 \pm 2.82^\circ$) (*P* = 0.0143) (Figure 6).

Discussion

This study showed that rigid fixation of the spinal column using a stainless steel rod stabilized the spinal column and reduced scoliosis, kyphosis, and scaffold malalignment. Impregnation of the PLGA scaffolds with radio-opaque barium sulfate allowed for visualization of the implant within the transected cord using radiographical analysis and 3-dimensional-MRM technology allowed measurement of scaffold malalignment.

Development of kypho-scoliosis in animals who have not received spinal stabilization may compromise the alignment of the implanted scaffold, thereby impairing bridging of the scaffold with the transected spinal cord and reducing the potential to promote axonal regeneration. Spinal fixation across the thoracic and lumbar segments of the spinal column in rats is a challenge. Internal fixation using 2 stainless steel rods shaped to accommodate the physical curvature of the spinal column has been demonstrated.¹⁴ This resulted in a decrease in

meningeal scarring and secondary degeneration with less cavitation when compared to unfixed groups 14 days post transection.

Here we used a stainless steel rod, overlaying wires fastened to the dorsal spinous processes, to stabilize the spinal column. Radiographic analysis demonstrated prevention of scoliosis in animals with spinal fixation, while 3-dimensional-MRM analysis showed reduced malalignment of the scaffold. Sparing of as little as 5 to 10% of corticospinal axons is estimated for basic functional recovery after SCI.^{16,17} A correctly aligned scaffold loaded with therapeutic growth factors or cell types could potentially promote the regeneration required for functional improvements.^{2,11,12} Scaffold malalignment within the transected cord could potentially increase neural scar formation, which consists of many axonal inhibitory molecules such as chondroitin sulfate proteoglycans, semaphorins, and ephrins.^{18–20} Incorporation of the spinal fixation procedure, is therefore, important for this type of SCI model system. It allows assessment of the level of axonal regeneration occurring as a result of delivery of therapeutic factor(s), without hindrance from scaffold malalignment. The multichannel scaffold provides clearly defined regions in which to count axons and because all axons are transected at the time of injury, we can be certain that any regeneration seen is not as a result of untransected endogenous neurons.

This work represents an evolving animal model for severe SCI. The various aspects of the model emphasize the importance of the multidisciplinary research team; in this case including tissue engineers, polymer chemists, orthopedists, veterinarians, and neurosurgeons. Using a highly optimized model will greatly increase the potential of finding a therapy for restoring function to the injured cord.

Key Points

- Radio-opaque barium sulfate allowed for *in vivo* visualization of implanted scaffolds using radiographic analysis.
- MRM confirmed the scaffold alignment.
- Fixation of the spinal column reduced scaffold malalignment within the transected cord.
- Fixation of the spinal column prevented scoliosis and reduced kyphosis.

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Figure 1.

Surgical procedure demonstrating scaffold placement and fixation of the spinal column.

Laminectomy performed at vertebral level T9/T10 exposing the spinal cord (A,). After implantation of the scaffold into the transected cord, holes are drilled through the dorsal spinous processes and wires are passed through the middle, over which a steel rod is laid (B,). The steel rod is fastened in place using the wires (C,).

Figure 2. Lateral radiographs showing progressive scoliosis in the unfixed rat. Radiographs were taken immediately (A,) 2 weeks (B,) and 4 weeks (C,) post implantation of the scaffold into the transected cord. Arrows denote location of the scaffold at each time-point.

Figure 3. Radiographs showing the degree of scoliosis in the fixed rat. Anterior/posterior radiograph showing the well-aligned scaffold at 4 weeks after spinal stabilization with a single steel rod (A.). Lateral radiograph showing well aligned spine 4 weeks after implantation of the scaffold with minimum scoliotic deformity after spinal fixation (B.).

Figure 4. Scoliosis and kyphosis measurements 4 weeks after scaffold implantation into the transected cord. Graphs show the degree of scoliosis (**A**,) and kyphosis (**B**,) at 4 weeks. Error bars represent standard error of the mean (n = 9). * $P = 0.0004$.

Figure 5. Effect of spine stabilization on scaffold alignment. 3-dimensional-magnetic resonance microscopy (MRM) coronal (A and B,) and axial (C and D,) images 4 weeks after scaffold implantation into the transected cord with (A and C,) or without (B and D,) spinal fixation.

Figure 6.

Degree of scaffold malalignment 4 weeks post implantation into the transected cord. Graph represent the average degree of malalignment of the scaffold in coronal and sagittal planes in the unfixed *versus* fixed spinal column groups. Error bars represent standard error of the mean (n = 9). * $P = 0.0143$.